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Age-Dependent Changes in Gastrointestinal Transport and Retention of Particulate Manganese Oxide in the Rat

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16. ABSTRACT

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AGE-DEPENDENT CHANGES IN GASTROINTESTINAL TRANSPORT AND RETENTION OF PARTICULATE MANGANESE OXIDE IN THE RAT

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Translocation of inhaled particulates from the nasopharynx and upper tracheobronchial area to the gastrointestinal tract is a major route of exposure for particles with a mass median diameter of greater than 1 μm. Previous studies in this laboratory with particulate Mn_3O_4 have shown that preweanling rats have substantially higher tissue Mn concentrations than similarly treated adults, indicating possible differences in uptake or elimination or both. This study was conducted to evaluate changes in gastrointestinal movement and retention of particulate Mn_3O_4 in the preweanling and weaned rat. ^{85}Sr-labeled microspheres were used to evaluate gastrointestinal transit rate (TR), while particulate Mn_3O_4 was used to evaluate particulate retention at selected ages. The results show that stomach retention time in the preweanling is at least twice that of the postweanling (90 min versus 42 min). In general, intestinal TR was not different in any of the ages evaluated, while transit time increased as intestinal length increased. Analysis of the Mn data demonstrated that the preweanling rat had a two-component retention curve with half-times of between 2 and 6 h for the short component and of between 24 and 26 h for the long component. In the postweanling rat, only one component was identified, with a half-time of between 2 and 5 h.

INTRODUCTION

A number of factors affect the body's reaction to the ingestion of pollutants. One of these is age, in that the mechanism that allows the gastrointestinal (GI) tract to selectively absorb substances is not completely developed at birth in most mammals studied (Clark, 1959). The mechanism for absorption of macromolecules by the neonatal intestinal epithelium is pinocytosis. This activity of the intestinal columnar absorptive cells is an unselective mechanism for uptake. Properties that determine the ability of a substance to penetrate the membrane barrier by pinocytosis include particle size, duration of contact of the luminal contents with the intestinal surface, solubility, charge, and susceptibility of the substance to extracellular digestion. Movement of ma-

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terial through the GI tract of the immature rat is reported to be substantially slower than in the adult, resulting in an increase in the average duration of contact of the luminal contents with any given length of the GI tract (Sikov and Mahlum, 1967; Varga, 1976). The GI tract of the rat undergoes many maturational changes during the first 2–3 wk, after birth (Halliday, 1956; Clarke and Hardy, 1969; Herbst and Sunshine, 1969; Baintner and Veress, 1970; Varga, 1976; Henning, 1979). The slower movement of material through the GI tract, the ability of the neonatal intestinal epithelium to incorporate (by pinocytosis) macromolecules (Clark, 1959), and the slower migration rates of epithelial cells toward the tips of the villi (Koldovsky et al., 1966) all markedly increase the residence time, and therefore the absorption potential, of toxicants in the preweanling animal. Previous work in this laboratory (Rehnberg et al., 1980, 1981) has demonstrated that particulate Mn_3O_4 exposure in rats resulted in maximal tissue Mn concentrations during the period around weaning (21–24 d of age).

This study was designed to investigate the rate of movement of material through the GI tract of the pre- and postweanling rat and the retention of particulate Mn_3O_4 in the GI tract of the rat during this period of rapid change.

MATERIALS AND METHODS

This study was conducted in two parts. Study one was conducted to determine the rate of movement of bulk indigestible material through the GI tract. Study two was conducted to determine the residence time of particulate Mn_3O_4 in the GI tract of the pre- and postweanling rat.

In study one, pregnant and 21-d-old Sprague-Dawley rats were obtained from Charles River Laboratories, Wilmington, Mass. On d 2 postpartum, litters were culled to 8 pups, 4 males and 4 females. Pups were weaned at 24 d of age, and on d 3, 12, 24, and 30 postpartum, equal numbers of male and female pups were gavaged with 0.5–1.0 μCi strontium-85- (^{85}Sr-) labeled polystyrene microspheres (9 ± 1 μm) in 0.1 ml saline (3M Company, Minneapolis, Minn.). Prior to administration, 0.1-ml aliquots of the dosing solution containing the microspheres in saline were counted to determine the amount of radioactivity. Animals were gavaged, the feeding needle and syringe rinsed with distilled water, and the rinse returned to the original counting vial. The remaining activity was determined, and the difference was taken as the dose received by the animal. Rats were not fasted prior to and were not allowed food after administration of the microspheres. Pups were kept warm during the period of food deprivation.

At selected times (1–10 h) after administration of the microspheres, six nonlittermate rats from each control and dose group were killed, and the lungs, stomach, small intestine, cecum, and large intestine

were removed. Lungs were taken to determine whether the microspheres had been correctly administered. When significant amounts (greater than 1% of the administered dose) were found in the lungs, the animal was excluded from the study. Lengths of the small and large intestine were measured, and the small intestine was divided into four sections of equal length. Radioactivity in the stomach and each quarter of the small intestine was measured by gamma counting.

In the second study, timed-pregnant Sprague-Dawley rats were obtained from Charles River Laboratories, Wilmington, Mass. They were housed in individual cages with ad libitum access to standard laboratory chow (Purina), containing 50 mg Mn/kg diet (as $MnSO_4$), and tap water. Pups were delivered naturally and each litter was adjusted to 8 pups (4 males and 4 females per litter) within 48 h after parturition. On d 12 or 24 postpartum, animals were administered 3–4 μl/g body weight (BW) of a 50% sucrose suspension containing 21.4 μg Mn (as Mn_3O_4)/μl. The average Mn_3O_4 particle diameter was 1.02 μm, with 78% of the particles less than 1.8 μm in diameter (Rehnberg et al., 1980).

At selected intervals (1–168 h) after administering the Mn_3O_4, 6–8 rats from the control and the same number from the Mn-treated group were killed and the stomach and small intestine were removed. The length of the small intestine was measured and then divided into four equal segments. The stomach and small-intestinal segments were then washed with distilled water to remove the luminal contents, patted dry, and weighed. Tissues were placed in plastic vials and stored at −60°C until assayed. Manganese assays on tissues from treated and control animals were done using flame atomic absorption spectrophotometry. The analytical method used was the NIOSH p and CAM-173. The accuracy of the analytical method was confirmed by assaying NBS bovine liver containing 10.3 ± 1.0 ppm Mn. The mean concentration of Mn in the NBS standard was found to be 10.2 ± 0.6 ppm by the above procedure. Total Mn content of each segment in control and treated animals was determined. Total Mn in each segment of treated animals was then corrected by subtracting the average amount of endogenous Mn present in control animals of the same age. This difference represented the Mn present in the GI tract due to the intubated dose of Mn_3O_4. Data were then expressed as a percentage of the administered dose and were analyzed with a computer-based statistical package (Helwig and Council, 1979) containing a nonlinear (NLIN) fitting procedure. The Marquardt regression procedure was used to fit the transformed data with a convergence criteria of 0.001 initially to a biexponential model of the form of $Y = C_1 \exp[(-B_1)X] + C_2 \exp[(-B_2)X]$. In this equation, Y is the Mn content (as percent of administered dose), C_1 is the intercept of the initial slope, B_1 is the initial slope component, X is the time the sample was obtained post dosing, C_2 is the intercept of the slow component, and B_2 is the slow slope component. When the biexponential

fitting was not significant ($p > 0.05$), data were fitted to the monoexponential model. The data from each intestinal component was fitted using the peak absorption as an initial value and all subsequent values above the minimal detectable limit (0.003% of the administered dose). Data from both studies were analyzed for sex differences. Since no sex differences were found, all analytical procedures used pooled data.

RESULTS

The ^{85}Sr contents of the individual segments of the rat gastrointestinal tract expressed as a cumulative percentage of the administered dose are presented in Table 1, and lengths of the small and large intestine are presented in Table 2.

The ^{85}Sr activities in a segment and in all proximal segments were added and expressed as a cumulative percent of the administered

TABLE 1. ^{85}Sr Content of Segments of the Gastrointestinal Tract Expressed as a Cumulative Percent of the Administered Dose (Study One)

Age (d)	Hours post dosing	N	Stomach	Intestinal segment 1	2	3	4
3	1	6	66	75	86	99	—
	2	6	41	45	50	66	95
	3	6	35	37	43	54	70
	4	6	13	14	15	20	40
	6	6	—	—	—	—	12
12	0.5	4	92	93	—	—	—
	1	4	89	91	93	98	—
	2	4	76	78	80	92	—
	4	6	29	31	33	43	100
	5	6	29	33	41	47	68
	6	6	21	24	28	37	65
	8	3	3	3	4	6	36
	10	6	—	—	—	—	17
24	0.5	4	88	97	—	—	—
	1	4	68	74	95	—	—
	2	4	52	58	75	100	—
	4	10	15	16	23	32	100
	5	6	17	18	21	45	82
	6	10	18	19	23	32	63
	8	4	5	5	6	8	11
	10	4	—	—	—	—	7
30	1	6	52	62	85	100	—
	2	6	25	30	43	73	100
	4	6	19	21	28	39	63
	6	6	5	7	9	13	26
	9	6	—	—	—	2	6

TABLE 2. Length of Small and Large Intestine in Young Rats (Study One)[a]

Age (d)	N	Body weight (g)	Small intestine (cm)	Large intestine (cm)
3	41	10.5 ± 1.0	33.6 ± 2.8	4.2 ± 0.8
12	56	37.6 ± 3.6	50.8 ± 4.3	7.1 ± 1.0
24	88	81.4 ± 10.2	97.6 ± 7.8	12.9 ± 1.8
30	48	111.2 ± 13.4	95.6 ± 7.4	14.2 ± 1.9

[a] Mean ± SD.

dose. These percentages were transformed into the logarithm and fitted to a linear model [log percent remaining in a segment and proximal segments = b_0 b_1 (time)]. The intercepts (b_0) and slopes (b_1) of the fitted regression lines are shown in Table 3. Transit half-times ($T_{1/2}$) were calculated from the fitted statistics as described by Varga (1976). Transit time (TT) is the difference between the $T_{1/2}$ of one segment and its proximal segment. Transit rate (TR) for each segment is in centimeters per minute.

The calculated GI transit parameters are presented in Table 4. Stomach $T_{1/2}$ increased from 1.5 h in 3-d-old pups to 2.5 h in 12-d-old pups and then dropped to 1.5 and 0.75 h in 24- and 30-d-old rats, respectively. Transit time (TT) through the proximal half (segments 1 + 2) of the small intestine increased between 3- and 30-d-old rats, while approximately doubling in the fourth segment during the same age range. In the 30-d-old rat, TT in the distal half decreased approximately 30% from the rates observed in the 24-d-old animals. Transit rates were not different in the proximal half (segments 1 + 2) and in segment 3 at any of the ages measured. However, TR values in segment 4 of the 24- and 30-d-old animals were 2–3 times higher than those observed in the 3- and 12-d-old animals. From the limited data collected on the large intestine and cecum, transit times in the preweanling rat appear to be shorter than in the 30-d-old postweanling rat.

This first study compares intestinal transit of nonfasted pre- and postweanling rats. The most significant difference between the pre- and postweanling animals was in stomach-emptying $T_{1/2}$ and TR of material in the ileal segment (segment 4) of the small intestine. The longer residence time of material in this segment of the small intestine in the preweanling rat is of interest because of the potential for increased absorption of particulates by the columnar epithelial cells.

The second phase of this study was conducted to determine the residence time of particulate Mn_3O_4 in the GI tract of pre- and postweanling rats. It is understood that most of the Mn_3O_4 retained in the wall of the small intestine is eventually sloughed into the lumen with senescent epithelial cells and excreted. However, the substantial in-

TABLE 3. Intercepts and Slopes of the Fitted Regression Lines[a,b] Calculated from Data in Table 1 (Study One)

Age (d)	Variable	Stomach	Intestinal segment			
			1	2	3	4
3	Intercept	4.87 ± .21	5.02 ± .20	5.15 ± .20	5.29 ± .16	5.84 ± .20
3	Slope	−0.581 ± .069	−0.606 ± .067	−0.616 ± .066	−0.563 ± .054	−0.572 ± .048
12	Intercept	4.92 ± .23	4.93 ± .23	5.06 ± .30	5.12 ± .24	6.05 ± .29
12	Slope	−0.399 ± .049	−0.377 ± .049	−0.373 ± .061	−0.324 ± .049	−0.348 ± .044
24	Intercept	4.67 ± .44	4.77 ± .37	5.16 ± .41	5.87 ± .46	6.85 ± .33
24	Slope	−0.495 ± .094	−0.479 ± .079	−0.492 ± .082	−0.499 ± .087	−0.526 ± .051
30	Intercept	4.23 ± .31	4.42 ± .29	4.81 ± .27	5.40 ± .34	5.68 ± .29
30	Slope	−0.450 ± .083	−0.453 ± .076	−0.472 ± .072	−0.511 ± .058	−0.448 ± .049

[a] Log percent remaining in a segment and proximal segment = b_0(intercept) + b_1(slope) (time).
[b] Mean ± standard error of the estimate, 30–46 animals/group.

TABLE 4. Gastrointestinal Transit Half-Times ($T_{1/2}$)[a], Transit Times (TT)[a], and Transit Rates (TR)[a] in the Young Rat (Study One)

Age (d)		Stomach	Intestinal segment						
			1	2	1 + 2	3	4	3 + 4	1 + 2 + 3 + 4
3	$T_{1/2}$	99	110	122	—	147	203	—	—
3	TT	—	11	12	23	25	56	81	104
3	TR	—	0.76	0.70	0.73	0.34	0.15	0.21	0.32
12	$T_{1/2}$	152	162	185	—	224	369	—	—
12	TT	—	10	23	33	39	145	184	217
12	TR	—	1.27	0.55	0.77	0.33	0.09	0.14	0.24
24	$T_{1/2}$	91	107	152	—	236	335	—	—
24	TT	—	16	45	61	84	99	183	244
24	TR	—	1.52	0.54	0.80	0.29	0.25	0.27	0.40
30	$T_{1/2}$	42	68	114	—	175	237	—	—
30	TT	—	26	46	72	61	62	123	195
30	TR	—	0.92	0.52	0.66	0.39	0.39	0.39	0.49

[a] Units of $T_{1/2}$ and TT are in minutes and TR in centimeters per minute.

creases in tissue Mn concentrations reported previously (Rehnberg et al., 1980, 1981) were evidence that a significant amount of Mn was absorbed through the gut wall and subsequently transferred to tissue.

Manganese content of individual segments of the GI tract, expressed as a percent of the administered dose at various times post dosing, is shown in Table 5. The initial uptake of Mn by the small intestine was decidedly greater in the 24-d-old when compared to the 12-d-old rat. In the 24-d-old rat, peak levels of Mn in segments 1–3 were reached with 2 h after dosing and then dropped precipitously. By 24 h post dosing, the entire GI tract of the 24-d-old was relatively clear of Mn (less than 0.1% of the administered dose), while in the 12-d-old animal a similar condition was not reached until between 72 and 96 h post dosing. In the proximal half of the small intestine of the 12-d-old rat, the absorption peak was approximately 8 h post dosing. Manganese levels in the distal portion in this age group, especially in segment 4, plateaued between 8 and 24 h post dosing and declined thereafter. Substantial amounts of Mn were still present in the small intestine of the younger animal 72 h after gavage, principally in the distal portion.

TABLE 5. Manganese Content of the Individual Segments Expressed as a Percentage of the Administered Mn_3O_4 Dose (Study Two)

Age (d)	Hours post dosing	N	Stomach	Intestinal segment 1	2	3	4
12	1	6	0.541	0.174	0.093	0.011	0.001
	2	6	0.423	0.162	0.106	0.065	0.003
	4	6	0.322	0.175	0.148	0.091	0.064
	8	6	0.191	0.279	0.268	0.158	0.167
	16	6	0.069	0.100	0.107	0.154	0.166
	24	6	0.099	0.119	0.124	0.126	0.176
	48	6	0.037	0.050	0.041	0.061	0.080
	72	6	0.010	0.023	0.023	0.048	0.045
	96	6	[a]	0.013	0.006	0.018	0.018
	168	6	[a]	0.015	0.014	0.009	0.013
24	1	6	0.692	0.370	0.257	0.042	0.005
	2	6	0.932	0.614	0.699	0.588	0.072
	4	6	0.727	0.322	0.292	0.465	0.048
	8	6	0.127	0.103	0.101	0.121	0.078
	16	6	0.028	0.041	0.018	0.036	[b]
	24	6	0.030	0.017	0.011	0.013	0.017
	48	6	0.011	0.005	0.005	0.010	0.007
	72	6	0.003	0.004	[a]	0.008	0.002
	96	6	0.005	0.005	0.004	0.003	0.003
	168	6	0.026	0.003	[a]	[a]	[a]

[a] Below limit of dectability.
[b] Samples contaminated.

TABLE 6. Fit Statistics and $T_{1/2}$ for Manganese Retention (Study Two)

Statistic	Stomach	Intestinal segment 1	2	3	4	Overall
			Age 12 d[a]			
Intercept (C_1)	0.504	−0.217	−0.364	−0.276	−0.472	−0.568
	±0.053	±0.192	±0.234	±0.028	±0.160	±0.329
Slope (B_1)	0.248	0.283	0.265	0.223	0.123	0.245
	±0.055	±0.430	±0.246	±0.047	±0.042	±0.283
$T1_{1/2}$ (h)	2.8	2.5	2.6	3.1	5.6	14.7
Intercept (C_2)	0.142	0.333	0.372	0.241	0.415	1.243
	±0.058	±0.222	±0.266	±0.030	±0.170	±0.364
Slope (B_2)	0.029	0.046	0.051	0.026	0.32	0.0311
	±0.014	±0.029	±0.0.031	±0.004	±0.009	±0.010
$T2_{1/2}$ (h)	23.9	15.1	13.7	26.7	21.5	107.3
			Age 24 d[b]			
Intercept (C_1)	1.59	1.12	1.52	0.94	0.16	4.88
	±0.19	±0.06	±0.010	±0.08	±0.02	±0.225
Slope (B_1)	0.24	0.30	0.39	0.21	0.093	0.254
	±0.04	±0.02	±0.03	±0.03	±0.012	±0.015
$T1_{1/2}$ (h)	2.9	2.3	1.8	3.3	7.5	18.8

[a] $y = C_1 \exp(-B_1 t) + C_2 \exp(-B_2 t)$.
[b] $y = C_1 \exp(-B_1 t)$; second component not significant ($p > 0.05$).

In the 24-d-old no appreciable amounts of Mn remained in the GI tract 72 h after gavage.

The fit statistics and the calculated $T_{½}$ values for particulate Mn in the GI tract of the pre- and postweanling rat are presented in Table 6. The retention pattern of Mn_3O_4 in the suckling rat was decidedly different from that of the 24-d-old weanling (Figs. 1, 2, and 3). The retention of Mn by individual segments of the GI tract of the 12-d-old suckling rat showed a two-component curve. One component (C_1, B_1) was rapid and resembled the retention curve of the 24-d-old weanling. The second curve (C_2, B_2), present only in the 12-d-old, showed a much slower decrease in Mn content with a considerably longer half-life. The overall $T_{½}$ of Mn in the GI tract of the preweanling rat, calculated from the total Mn content, represented by the first component of the retention curve was 14.7 h, while in the 24-d-old weanling it was 18.8 h. The $T_{½}$ of the second component (C_2, B_2) in the preweanling rat was 107.3 h and represents a significant exposure component for Mn in this age group.

DISCUSSION

These results on transit half-times, transit times, and transit rates are in general agreement with those of Varga (1976). However, stomach half-times and the half-times in the proximal intestinal segments are

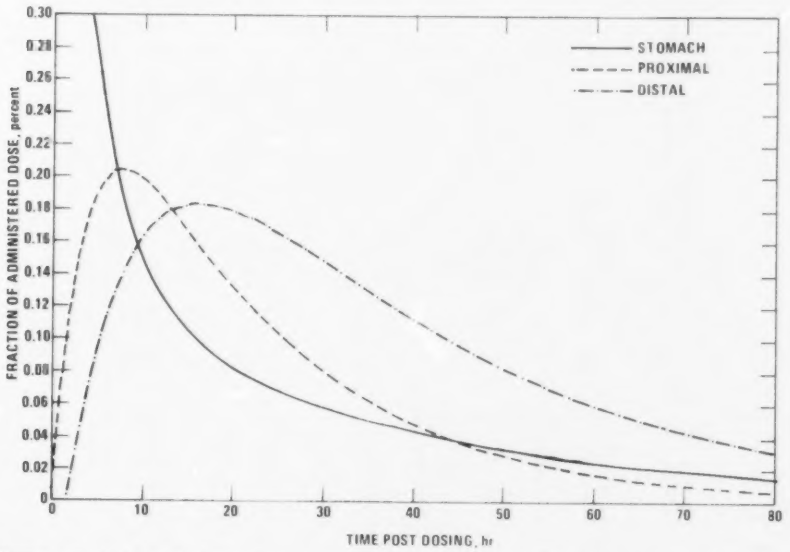

FIGURE 1. Gastrointestinal manganese retention fitted curves for the 12-d-old rat. Curves generated from the fit statistics in Table 6.

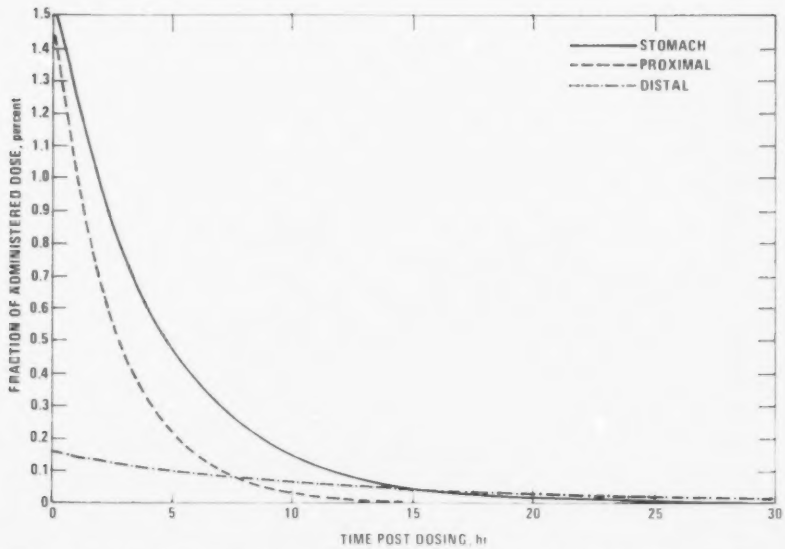

FIGURE 2. Gastrointestinal manganese retention fitted curves for the 24-d-old rat. Curves generated from the fit statistics in Table 6.

longer than those reported by Varga (1976), while the overall half-times in the small intestine are comparable. The differences are most likely due to the fact that Varga used fasted rats in his study while we used rats that were allowed food and water up to the time of dosing.

In previous studies (Rehnberg et al., 1980, 1981), it was found that chronic exposure of preweanling rats to particulate Mn_3O_4 resulted in abnormally high tissue concentrations of Mn, while postweanling rats with similar chronic exposure had only small increases in tissue Mn. These high tissue concentrations of Mn in the preweanling indicated increased penetration of the intestinal membrane, transfer to the circulation, and subsequent transfer to tissue. Intestinal absorption and transfer to circulation appeared to be related to the concentration of particulates coming into contact with the mucosal surface, as evidenced by increased tissue concentrations relative to dose (Cahill et al., 1980).

Results presented here show that the movement of material through the GI tract, particularly through the ileal section of the small intestine, of the preweanling rat is significantly slower than in the weaned rat. In addition to the slower rate of movement of material, the 12-d-old retained a small but significant fraction of the ingested dose of Mn within the small intestine for a considerably longer period than in the post weanling rat. This increased residence time could be due in part to the difference in the rate of cellular proliferation in the pre- and

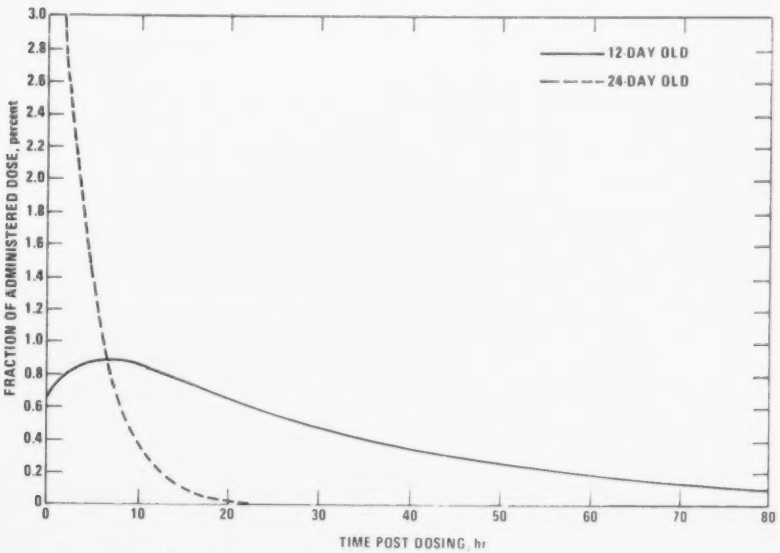

FIGURE 3. Overall gastrointestinal manganese retention fitted curves for the 12- and 24-d-old rat. Curves generated from the fit statistics in Table 6.

postweanling rat. The turnover rate of mucosal cells in the younger animal is about 20–25% of that of the weaned animal (Koldovsky et al., 1966; Clarke and Hardy, 1969), so that considerable intracellular digestion may occur before the cells are sloughed into the intestinal lumen. The younger animal also does not begin biliary excretion of Mn until about postnatal d 17 (Miller et al., 1975), another factor contributing to the increased tissue levels. Previous studies with exposures to high doses of Mn_3O_4 have shown a decreased percentage of liver Mn with much higher levels in other soft tissues, suggesting that the liver becomes saturated and can no longer extract Mn from circulation, allowing more Mn to become available to other organs (Rehnberg et al., 1981; Cahill et al., 1980).

Summarizing the data presented in this study, the following points are identified.

1. Stomach half-time is longer in the 3- to 24-d-old rat than in the postweanling (30-d-old) rat.
2. No appreciable differences in transit rate were seen in the proximal intestinal segment at any of the ages evaluated. Since the length of the small and large intestine increased from roughly 38 to 110 cm during the period of evaluation, this resulted in an age-related increase in the residence time of the intestinal contents.

3. Transit rate in the distal intestinal segments in the younger rats (3 and 12 d old) was slower than in the older rats (24 and 30 d old) tested.
4. With Mn exposure to the 12-d-old rat, data analysis demonstrated a two-component retention curve for each intestinal segment, ranging from 2.5 to 5.6 h for the short component and from 13 to 26 h for the long component. In the 24-d-old rat only 1 retention component was identified, ranging from 2 to 7.5 h. These results indicated an overall half-time of roughly 27 h in the 12-d-old rat and of roughly 2.7 h for the 24-d-old rat.

In addition to its significance in terms of the uptake and subsequent exposure to the relatively insoluble Mn_3O_4, this study presents an important consideration in the assessment of preweanling exposure to many potential particulate toxicants.

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